Applicants: Akioy YAMADA et al.

IN THE SPECIFICATION:

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Please amend the specification beginning at page 6, line 7 and ending a page 17, line 6, as follows:

The present invention relates to a method for screening DNA encoding proteins having the activity of improving environmental stress tolerance wherein candidate cDNA derived from cDNA library is introduced into host cells, the obtained transformed cells are cultured under the conditions where the host cells cannot substantially grow, the clones grown after culturing are selected, and the candidate cDNA introduced from the selected clones is isolated (1), a method for screening DNA encoding proteins having the activity of improving environmental stress tolerance wherein candidate cDNA derived from cDNA library is introduced into host cells, the obtained transformed cells are cultured under conditions where the host cells cannot substantially grow, the clones grown after the culturing are selected, candidate cDNA is isolated from the selected clones, the isolated candidate cDNA is introduced into the isolated cDNA, the mutant cDNA is introduced into host cells, and the process of selecting is repeated one or more times under stringent conditions of selecting mutant cDNA than the selecting condition (2), the a method for screening according to (1) or (2) one of the above, wherein the environmental stress is one or more of chemical substance stress, high temperature stress, low temperature stress, freezing stress, drought stress, ozone stress, ultraviolet stress, radiation stress, or osmotic pressure stress (3), the a method for screening according to one of the above (3), wherein the chemical substance stress is salt stress (4), the a method for screening according to any one of (1) to (4) the above, wherein the host cell is a coliform (5), the a method for screening according to one of the above (5), wherein the coliform is SOLR strain (6), the a method for screening according to any one of the above (1) to (6), wherein an environmental condition where host cells cannot substantially grow is 350mM or more of salt concentration (7).

The present invention also relates to DNA encoding proteins having the activity of improving environmental stress tolerance wherein the DNA is obtained according to any one of the above (1) to (7) (8), DNA encoding proteins having the activity of improving environmental stress

tolerance according to one of the above (8), wherein the environmental stress is one or more of stresses selected from chemical substance stress, high temperature stress, low temperature stress, freezing stress, drought stress, ozone stress, ultraviolet stress, radiation stress, or osmotic pressure stress (9), DNA encoding proteins having the activity of improving the environmental stress tolerance according to 9 as described above, wherein the chemical substance stress is salt stress (10), DNA encoding proteins having the activity of improving the environmental stress tolerance according to any one of (8) to (10), wherein the proteins having the activity of improving the environmental stress tolerance are derived from plants (11), DNA encoding proteins having the activity of improving the environmental stress tolerance according to (11), wherein the plant is Bruguiera sexangla, Avicennia marina, Sueada japonica, Salsola komarovii, or Mesembryanthemum crystallinum (12), DNA encoding proteins according to any one of the following (a) to (c): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 2, (b) a protein comprising a sequence of amino acids having 70% or more of homology with the sequence of amino acids shown in Seq. ID No. 2, and having the activity of tolerance at least against salt stress, (c) a protein having a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 2, and having the activity of improving tolerance at least against salt stress (13), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 1, or its complementary sequence (14), DNA hybridized with the this DNA according to (14) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (15), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 4, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 4, and having the activity of improving tolerance at least against salt stress (16), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 3 or its complementary sequence (17), DNA hybridized with the this DNA according to (17) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (18), DNA encoding proteins according to any one of the

following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 6, (b) a protein comprising the sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 6, and having the activity of improving tolerance at least against salt stress (19), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 5 or its complementary sequence (20), DNA hybridized with the this DNA according to (20) under stringent conditions, and encoding proteins comprising the activity of improving tolerance at least against salt stress (21), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 8, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 8, and having the activity of improving tolerance at least against salt stress (22), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 7 or its complementary sequence (23), DNA hybridized with the this DNA according to (23) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (24), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising a sequence of amino acids shown in Seq. ID No. 10, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 10, and having the activity of improving tolerance at least against salt stress (25), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 9 or its complementary sequence (26), DNA hybridized with the this DNA according to (26) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (27), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein having the sequence of amino acids shown in Seq. ID No. 12, (b) a protein having a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 12, and having the activity of improving tolerance at least against salt stress (28), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 11 or its complementary sequence (29), DNA hybridized with the this DNA according to (29) under

stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (30), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein having the sequence of amino acids shown in Seq. ID No. 14, (b) a protein having a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 14, and having the activity of improving tolerance at least against salt stress (31), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 13 or its complementary sequence (32), DNA hybridized with the this DNA according to (32) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (33), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 16, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 16, and having the activity of improving tolerance at least against salt stress (34), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 15 or its complementary sequence (35), DNA hybridized with the this DNA according to (35) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (36), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 18, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 18, and having the activity of improving tolerance at least against salt stress (37), DNA having part or whole of the sequence of bases shown in Seq. ID No. 17 or its complementary sequence (38), DNA hybridized with the this DNA according to (38) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (39), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 20, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 20, and having the activity of improving tolerance at least against salt stress (40), DNA comprising

part or whole of the sequence of bases shown in Seq. ID No. 19 or its complementary sequence (41), DNA hybridized with the this DNA according to (41) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (42), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 22, (b) a proteins comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 22, and having the activity of improving tolerance at least against salt stress (43), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 21 or its complementary sequence (44), DNA hybridized with the this DNA under stringent conditions according to (44), and encoding proteins having the activity of improving tolerance at least against salt stress (45), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 24, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 24, and having activity of improving tolerance at least against salt stress (46), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 23 or its complementary sequence (47), DNA hybridized with the this DNA according to (47) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (48), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 26, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 26, and having the activity of improving tolerance at least against salt stress (49), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 25 or its complementary sequence (50), DNA hybridized with the this DNA according to (50) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (51), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 28, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted,

substituted, or added in the sequence of amino acids shown in Seq. ID No. 28, and having the activity of improving tolerance at least against salt stress (52), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 27 or its complementary sequence (53), DNA hybridized with the this DNA according to (53) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (54), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 30, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in. ID No. 30, and having the activity of improving tolerance at least against salt stress (55), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 29 or its complementary sequence (56), DNA hybridized with the this DNA according to 56 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (57), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 32, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 32, and having the activity of improving tolerance at least against salt stress (58), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 31 or its complementary sequence (59), DNA hybridized with the this DNA according to 59 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (60), DNA encoding proteins according to any one of the following (a) or (b): (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 34, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 34, and having the activity of improving tolerance at least against salt stress (61), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 33 or its complementary sequence (62), DNA hybridized with the this DNA according to (62) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (63). DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein comprising the sequence of amino acids shown in Seq. ID No. 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, or 64, (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, or 64, and having the activity of improving tolerance at least against salt stress (64), DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, or 63, or its complementary sequence (65), and DNA hybridized with the this DNA according to (65) under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress (66).

The invention also relates to a method for improving environmental stress tolerance, wherein the DNA <u>as described above according to any one of (8) to (66)</u> is used (67), the <u>a</u> method for improving the environmental stress tolerance according to 67, wherein the environmental stress is one or more of chemical substance stress, high temperature stress, low temperature stress, freezing stress, drought stress, ozone stress, ultraviolet stress, radiation stress, and/or osmotic pressure stress (68), and the <u>for example</u>, a method for improving environmental stress tolerance according to 68, wherein the chemical substance stress is salt stress (69).

The invention also relates to a protein comprising of the sequence of amino acids shown in Seq. ID No. 2 (70), a protein having 70% or more of homology with the sequence of amino acids shown in Seq. ID No. 2, and having the activity of improving tolerance at least against salt stress (71), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 2, and having the activity of improving tolerance at least against salt stress (72), a protein comprising the sequence of amino acids shown in Seq. ID No. 4 (73), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 4, and having the activity of improving tolerance at least against salt stress (74), a protein comprising the sequence of amino acids shown in Seq. ID No. 6 (75), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 6, and having

the activity of improving tolerance at least against salt stress (76), a protein comprising the sequence of amino acids shown in Seq. ID No. 8 (77), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 8, and having the activity of improving tolerance at least against salt stress (78), a protein comprising the sequence of amino acids shown in Seq. ID No. 10 (79), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 10, and having the activity of improving tolerance at least against salt stress (80), a protein comprising the sequence of amino acids shown in Seq. ID No. 12 (81), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 12, and having the activity of improving tolerance at least against salt stress (82), a protein comprising the sequence of amino acids shown in Seq. ID No. 14 (83), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 14, and having the activity of improving tolerance at least against salt stress (84), a protein comprising the sequence of amino acids shown in Seq. ID No. 16 (85), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 16, and having the activity of improving tolerance at least against salt stress (86), a protein comprising the sequence of amino acids shown in Seq. ID No. 18 (87), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 18, and having the activity of improving tolerance at least against salt stress (88), a protein comprising the sequence of amino acids shown in Seq. ID No. 20 (89), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 20, and having the activity of improving tolerance at least against salt stress (90), a protein comprising the sequence of amino acids shown in Seq. ID No. 22 (91), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 22, and

having the activity of improving tolerance at least against salt stress (92), a protein comprising the sequence of amino acids shown in Seq. ID No. 24 (93), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 24, and having the activity of improving tolerance at least against salt stress (94), a protein comprising the sequence of amino acids shown in Seq. ID No. 26 (95), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 26, and having the activity of improving tolerance at least against salt stress (96), a protein comprising the sequence of amino acids shown in Seq. ID No. 28 (97), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 28, and having the activity of improving tolerance at least against salt stress (98), a protein comprising the sequence of amino acids shown in Seq. ID No. 30 (99), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 30, and having the activity of improving tolerance at least against salt stress (100), a protein comprising the sequence of amino acids shown in Seq. ID No. 32 (101), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 32, and having the activity of improving tolerance at least against salt stress (102), a protein comprising the sequence of amino acids shown in Seq. ID No. 34 (103), a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 34, and having the activity of improving tolerance at least against salt stress (104), a protein comprising the sequence of amino acids shown in Seq. ID No. 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, or 64 (105), and a protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, or 64, and having the activity of improving tolerance at least against salt stress (106).

The present invention also relates to an antibody specifically bound to the protein according to

any one of (70) to (72) (107) described above, an antibody specifically bound to the protein according to any one of (73) to (104) (108), an antibody specifically bound to the protein according to any one of (105) or (106) (109), and the antibody according to any one of (107) to (109), wherein the antibody is for example, a monoclonal antibody (110).

The present invention also relates to a vector comprising the DNA encoding proteins <u>as</u> <u>described herein</u>, in <u>particular those</u> having the activity of improving tolerance against environmental stresses according to any one of (8) to (12) (111), a vector comprising the DNA according to any one of (13) to (15) (112), a vector comprising the DNA according to any one of (16) to (63) (113), and a vector comprising the DNA according to any one of (64) to (66) (114).

The present invention also relates to a transformed cell obtained by introducing the vector described above into a cell according to any one of (111) to (114) (115), in particular a transformed cell according to (115), wherein the host cell is a plant cell (116), and a method for producing proteins having the activity of improving environmental stress tolerance, wherein the transformed cells according to either of (115) or (116) cell is cultured, and recombinant proteins are collected from the transformed cells or the supernatant of the cultured liquid (117).

The present invention also relates to a transgenic plant obtained by introducing the DNA according to any one of (8) to (12) encoding proteins having the activity of improving environmental stress tolerance, and by dividing, proliferating and redifferentiating the plant cell (118), a transgenic plant obtained by introducing the DNA according to any one of (13) to (15) encoding proteins having the activity of improving environmental stress tolerance, and by dividing, proliferating and redifferentiating the plant cell (119), a transgenic plant obtained by introducing the DNA according to any one of (16) to (63) encoding proteins having the activity of improving environmental stress tolerance, and by dividing, proliferating and redifferentiating the plant cell (120), a transgenic plant obtained by introducing the DNA according to any one of (64) to (66) encoding proteins having the activity of improving environmental stress tolerance, and by dividing, proliferating and redifferentiating the plant cell (121), a transgenic plant obtained by introducing the vector described above according to any one of (111) to (114), and by dividing, proliferating and redifferentiating the plant cell (122), the for example a transgenic

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plant according to any one of (118) to (122), wherein the environmental stress is one or more of chemical substance stress, high temperature stress, low temperature stress, frenzying stress, drought stress, ozone stress, ultraviolet stress, radiation stress, and/or osmotic pressure stress (123), the for example a transgenic plant according to 123, wherein the chemical substance stress is salt stress (124), and a material for breeding derived from the transgenic plant according to any one of (118) to (122) (125).

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